

CCCXXII.—*An Experimental Study of Protective Colloids. Part I. The Influence of Concentration.*

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ALTHOUGH cases of colloidal protection have been known and used in industry from the earliest times, the first quantitative study of the phenomenon is to be found in the work of Zsigmondy (*Z. anal. Chem.*, 1901, **40**, 697), in which the relative protective action of a number of substances was measured by the weight required to inhibit the precipitation of a standard gold sol by a 10% sodium chloride solution. Later Billitzer (*Z. physikal. Chem.*, 1905, **51**, 129) found that in low concentrations gelatin gave anomalous results: when a small amount of gelatin was added to an electronegative colloid, precipitation by electrolytes was facilitated; on the other hand, gelatin in larger amounts hindered precipitation. In the case of an electropositive colloid, however, gelatin hindered precipitation at all concentrations.

Loeb (*J. Gen. Physiol.*, 1923, **5**, 479) investigated the concentrations of different salts required to precipitate suspensions of "gelatin-coated colloid particles" and found that they were practically identical with the concentrations of the same salts required for the salting out of gelatin from aqueous solutions. He stated that "neither effect showed any relation to the critical double layers surrounding the particles," and concluded that "protective colloids must be capable of forming a durable film on the surface of the suspended particles, and the molecules constituting the film must have a higher attraction for the molecules of the solvent than for each other; in other words, they must possess true solubility."

Further, Gann (*Koll.-Chem. Beih.*, 1916, **8**, 252) found that gelatin and other proteins precipitated a red gold sol, and later, Kermack and Wright (*Biochem. J.*, 1923, **17**, 635) studied the precipitation of a gum benzoin sol by gelatin over a wide range of values of p_H and found that precipitation occurred on the acid side of the isoelectric point of gelatin ($p_H = 4.7$) and protection on the alkaline side. Rideal (*Proc. Camb. Phil. Soc.*, 1924, **22**, 101) describes experiments in which very dilute gelatin solutions were added to a gold sol which was made acid or alkaline in separate experiments. Precipitation occurred at $p_H < 5$, and protection with the alkaline solutions. Rideal concludes from a discussion of these and other data that these anomalies in the behaviour of protective colloids are to be ascribed to idiosyncrasies in the specific adsorbing power for particular ions of the particles of sol and protective colloid.

Kermack and MacCullum (*Proc. Roy. Soc. Edin.*, 1924-25, **45**,

71) investigated the influence of gelatin on the stability of cholesterol suspensions over a wide range of acidities and found two zones of stable sols, one with high concentrations of gelatin in which the particles are positively charged, and the other with low concentrations in which the cholesterol particles are negatively charged.

Boutaric and Perreau (*Compt. rend.*, 1925, **181**, 511) have investigated the interaction of a number of negative colloids and protective colloids and have found that "in the majority of cases the addition of small quantities of protective colloids makes the sol more sensitive to the action of electrolytes; if larger quantities of colloid are added, the increase in sensitivity gradually diminishes to zero; if larger quantities of colloid are added, a protective action is more and more clearly shown; very large amounts bring about coagulation of the sol."

Many more observations of anomalous protective action and of sensitisation could be quoted, but, from a general survey of the literature, it appears probable that two distinct phenomena are concerned, according as the protective colloid is present in low or in high concentration. We have studied the effect of varying the concentrations both of protective colloid and of electrolyte; we have worked also with dispersoid colloids which could be obtained in considerable concentration, *e.g.*, arsenious sulphide and aluminium hydroxide, and have therefore been able to vary the concentration of the sol over a considerable range. It will be seen from the results which follow that, when the interaction of a system dispersoid colloid-emulsoid colloid-electrolyte is viewed broadly, all the systems studied show a remarkable similarity. Sensitisation and protection of the dispersoid colloid appear to be particular aspects of the reaction regarded as a whole and either may be brought about at will by a variation in concentration of the two types of colloid.

EXPERIMENTAL.

Arsenious sulphide sol was chosen as a typical negatively-charged colloid. It was prepared in the usual manner by making arsenious oxide into a paste and slowly adding it to boiling distilled water; after boiling for some time, the solution was allowed to cool, and saturated with sulphuretted hydrogen, which had been freed from acid vapours and spray by passage through two wash-bottles and a U-tube filled with cotton-wool; the sol was freed, as far as possible, from sulphuretted hydrogen by the prolonged passage of hydrogen and, after filtering, was bottled and left to "age" for several weeks. The concentration of the sols was determined by oxidation and precipitation as magnesium ammonium arsenate.

As an example of a positively-charged colloid, aluminium hydr-

oxide was used. This was prepared by dissolving aluminium chloride in dilute hydrochloric acid, adding a considerable quantity of distilled water, and precipitating the hydroxide by ammonia. The gelatinous precipitate was thoroughly washed, transferred to a flask, and boiled for some time with distilled water to which a few drops of $N/20$ -hydrochloric acid were occasionally added. After filtering, the sol was bottled and left to "age." Its strength was determined by coagulation with ammonium chloride and ignition of the precipitated hydroxide.

Coignet's "Gold Label" gelatin (ash content, 0.9%) was used as the first protective colloid. During the preliminary work, it became clear that the gelatin solutions prepared on different days varied in properties, and it was found necessary to standardise the preparation and to make up and mix the whole of the series of mixtures (usually 144) on the same day with the same gelatin solution. The method of preparation was to allow a weighed quantity of the gelatin to soak in distilled water for 24 hours; warming in a water-bath at about 60° for 10 minutes then made a satisfactory solution, which was diluted to the required strength.

Barium chloride, sodium chloride, and sodium sulphate were used as precipitating electrolytes. In each case it was found convenient to make up a series of stock solutions, the normalities of which decreased in geometrical progression.

The points of a system which had to be fixed first were (1) the concentration of electrolyte necessary to precipitate the sol in the absence of protective colloid, (2) the concentration of protective colloid required to cause mutual precipitation between the two colloids in the absence of electrolyte, and (3) the concentration of protective colloid beyond which mutual precipitation ceased. With these datum points, it was possible to judge approximately the most suitable ranges of concentration of electrolyte and protective colloid for investigation.

During preliminary work, the protective colloid was added to the sol and the precipitating electrolyte added later; this, however, gave unsatisfactory results owing to the masking of the protecting action by mutual precipitation phenomena. Afterwards the protective colloid was therefore always added to the electrolyte before mixing the three reactants together.

A number of test-tubes (usually 288) were steamed, washed out with distilled water, and left to drain thoroughly in a series of stands. When the test-tubes were dry, 5 c.c. of arsenious sulphide sol were pipetted into each in alternate rows. The sodium chloride and gelatin, giving together a total volume of 5 c.c. in each tube, were then pipetted into the remainder so that each horizontal row had

the same concentration of electrolyte and each vertical row the same concentration of gelatin. To effect mixing, the gelatin and sodium chloride were poured into the sol, the whole was poured back again and then finally transferred to the test-tube which originally contained the sol. After being corked up, the mixtures were allowed to stand for 24 hours. Precipitation was estimated by allotting numbers as follows: Complete precipitation, 4; nearly complete precipitation, 3; half-precipitation, 2; definite turbidity, 1; and no turbidity, 0.

The results then appeared as in Table I.

TABLE I.

Final conc. of electrolyte.	Precipitation number.											
	4	4	4	4	4	4	2	0	0	0	0	0
8N/5	4	4	4	4	4	4	2	0	0	0	0	0
4N/5	4	4	4	4	4	4	2	2	2	0	0	0
2N/5	4	4	4	4	4	4	4	2	1	1	0	0
N/5	4	4	4	4	4	4	4	4	4	2	0	0
N/10	4	4	4	4	4	4	4	4	4	4	2	0
N/20	4	4	4	4	4	4	4	4	4	4	2	0
N/40	1	4	4	4	4	4	4	4	4	4	2	0
N/80	0	2	4	4	4	4	4	4	4	4	2	0
N/160	0	0	4	4	4	4	4	4	3	1	0	0
N/320	0	0	2	4	4	4	4	4	2	0	0	0
N/640	0	0	0	4	4	4	4	4	2	0	0	0
N/1280	0	0	0	4	4	4	4	4	0	0	0	0
Final conc. of gelatin (%)	0.001	0.002	0.003	0.004	0.005	0.006	0.007	0.008	0.009	0.010	0.011	0.012

To save space and facilitate the comparison of the data obtained for different systems, full details are given for this sol only, and all the results are plotted in Fig. 1. In plotting the diagrams, results denoted by 3 are shown as complete precipitation (shaded areas), and those recorded as 1 are shown as no precipitation (clear areas).

The systems first investigated and shown in Figs. 1, 2, and 3 are:

FIG. 1.

- (a) Arsenious sulphide sol (0.290 g./l.) + gelatin + sodium chloride.
 (b) " " " (0.581 g./l.) + " + " "
 (c) " " " (1.173 g./l.) + " + barium chloride.
 (d) " " " (4.650 g./l.) + " + " "

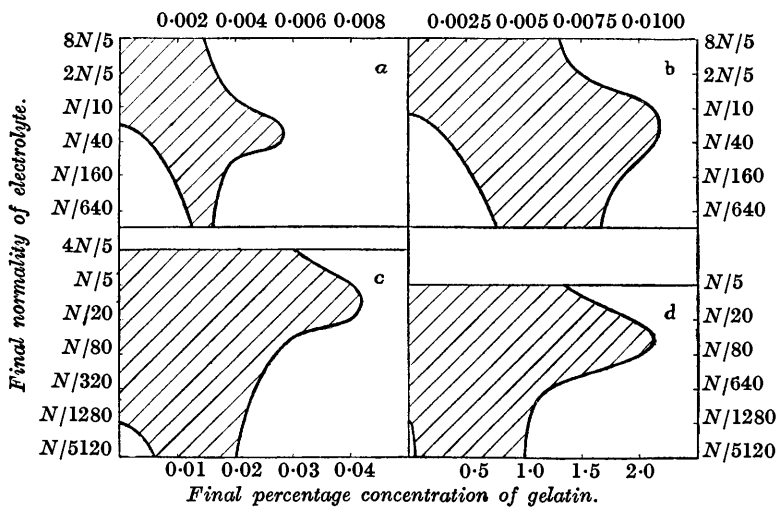
FIG. 2.

- (a) Aluminium hydroxide sol (0.770 g./l.) + gelatin + sodium chloride.
 (b) " " " (0.770 g./l.) + " + sodium sulphate.

FIG. 3.

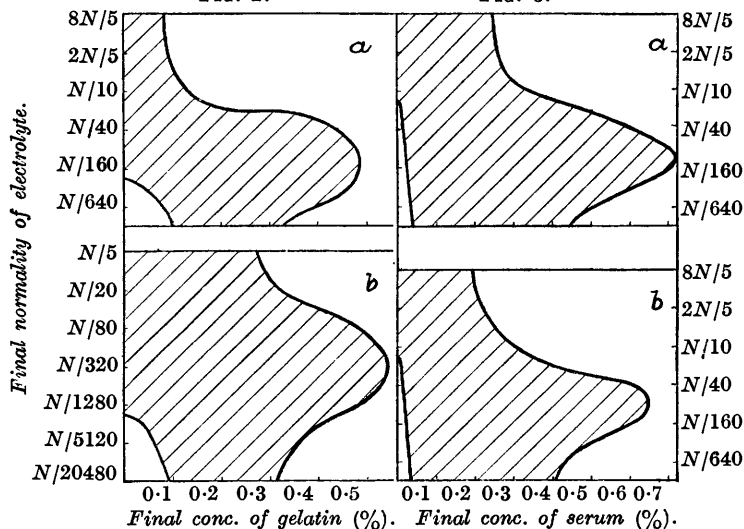
- (a) Arsenious sulphide sol (0.581 g./l.) + normal blood serum + sodium chloride.
 (b) " " " (0.581 g./l.) + syphilitic blood serum + sodium chloride.

FIG. 1.



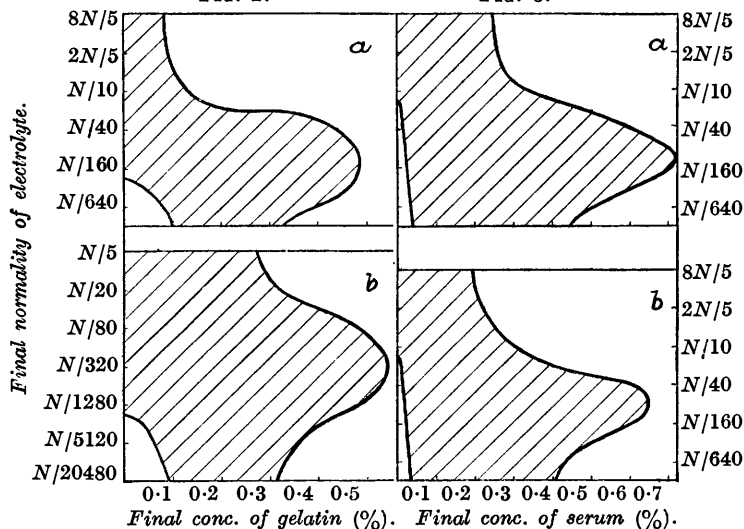
- (a) As_2S_3 + gelatin + NaCl. (b) As_2S_3 + gelatin + NaCl.
 (c) As_2S_3 + gelatin + BaCl_2 . (d) As_2S_3 + gelatin + BaCl_2 .

FIG. 2.



- (a) $\text{Al}(\text{OH})_3$ + gelatin + NaCl. (a) As_2S_3 + normal serum + NaCl.
 (b) $\text{Al}(\text{OH})_3$ + gelatin + Na_2SO_4 . (b) As_2S_3 + syphilitic serum + NaCl.

FIG. 3.



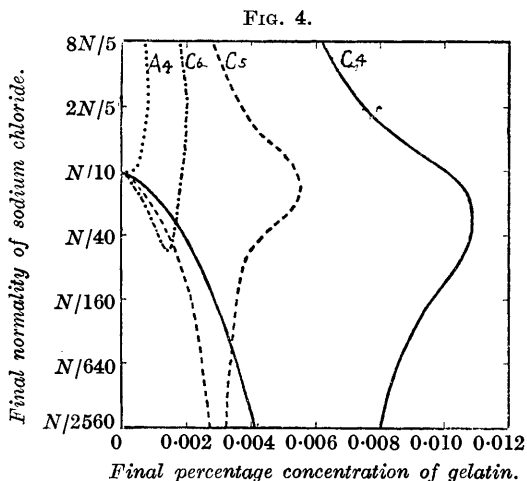
With one exception, all the experiments were carried out at room temperature. In system 1(d) the tubes were heated to 37° for 24 hours before reading, since such high concentrations of gelatin were

needed that a jelly was formed in many of the tubes at room temperature.

Discussion of Results.

We shall use the term "dispersoid sol" to designate the colloid the concentration of which is kept constant through a series of mixtures (arsenious sulphide, aluminium hydroxide) whilst the term "protective colloid" is used for the one the concentration of which is varied (gelatin, blood-serum).

It is immediately apparent from the diagrams that all the systems investigated show a great similarity in behaviour. The characteristic features are: (1) Small amounts of protective colloid produce a greater sensitivity towards electrolytes; (2) this sensitivity increases progressively until the two colloids precipitate one another even in



the absence of electrolyte; (3) at higher concentrations of protective colloid, a protective effect is found, but the curves exhibit a curious shape with a definite proboscis at certain concentrations of electrolyte.

These characteristic features are found in all the diagrams, although every possible variable has been changed. For the negative arsenious sulphide sols of varying concentrations, the same type of curve is given with univalent and bivalent positive precipitating ions and with gelatin and blood-serum as protective colloids. (Blood-serum was used in connexion with the Sachs-Georgi test; an account of further experiments in this reaction will be published shortly in another journal.) Similarly, the positive sol, aluminium hydroxide, with gelatin as protective colloid, gives the same type of curve with both uni- and bi-valent negative precipitating ions.

The principal effect of increasing the concentration of the sol

appears to be to shift the curve towards the region of greater concentrations. This is shown clearly in Fig. 4, which gives the curve for a series of sols obtained by diluting the strongest sols examined. An interesting quantitative relationship is revealed when the concentration of the sol is compared with the amount of gelatin which causes maximum precipitation. For this latter quantity, we have taken the concentration indicated by the middle point of the zone of precipitation by gelatin alone. As Table II shows, for three different sols the concentration of gelatin is roughly proportional to the concentration of the sol when the latter falls below about 1 g. per litre.

TABLE II.

Sol.	As ₂ S ₃ (g./l.).	Gelatin (g./l.).	Ratio Gelatin : As ₂ S ₃ .	Sol.	As ₂ S ₃ (g./l.).	Gelatin (g./l.).	Ratio Gelatin : As ₂ S ₃ .
A ₁	0.581	0.060	0.103	C ₁	4.650	5.03	1.13
A ₂	0.290	0.029	0.100			5.25	1.18
A ₃	0.145	0.015	0.103	C ₂	2.325	1.50	0.645
A ₄	0.072	?	—	C ₃	1.162	0.150	0.129
B ₁	1.173	0.13	0.111	C ₄	0.581	0.060	0.103
				C ₅	0.290	0.030	0.103
				C ₆	0.145	0.015	0.103

This result is somewhat surprising, since the sols were prepared under widely varying conditions. For sols A₁ and B₁, the whole of the arsenious oxide was dissolved in the water before passing sulphuretted hydrogen; to attain the concentration of sol C₁, however, it was necessary to add a paste of the oxide and water at intervals during the passage of the gas. Nevertheless, this sol on dilution gives precisely the same values for the amounts of gelatin which cause the maximum precipitation. Sols C₂, C₃, etc., were prepared by diluting C₁ and were allowed to "age" for 3 weeks before use. It appears, therefore, that a diluted arsenious sulphide sol on standing reaches a steady state in so far as precipitation is concerned. The numerical value of the ratio gelatin/arsenious sulphide is of interest. The maximum precipitation is produced by a quantity of gelatin that is roughly one-tenth of the weight of the arsenious sulphide present. This is in harmony with the view that the reaction is one between small aggregates of gelatin molecules and large aggregates of the sulphide.

The general effect of dilution of the dispersoid sol on the form of the diagram is shown in Fig. 4. The concentration of the sol has little or no influence on the amount of electrolyte which produces coagulation in the absence of gelatin. All the sols of arsenious sulphide are precipitated by *N*/10- to *N*/20-sodium chloride (Figs. 1a, 1b, and 4) and by *N*/1280-barium chloride (Figs. 1c and 1d).

The position of the "nose" varies systematically with the con-

centration of the sulphide; this is shown for the weaker sols in Fig. 4. Table III gives the position of the tip of the nose for the complete C-series of sols.

TABLE III.

Sol.	As ₂ S ₃ (g./l.).	Position of nose.		Sol.	As ₂ S ₃ (g./l.).	Position of nose.	
		NaCl conc.	Gelatin (g./l.).			NaCl conc.	Gelatin (g./l.).
C ₁	4.65	N/60	19.5	C ₄	0.581	N/30	0.108
C ₂	2.325	N/40	14.0	C ₅	0.290	N/15	0.055
C ₃	1.162	N/30	0.75	C ₆	0.145	2N/5	0.021

The most remarkable feature of these results is the great extension of the zone of precipitation into solutions of high gelatin content in sols C₁ and C₂.

It appears probable from our results that the behaviour of protective colloids in general may be represented by a diagram of the form shown in Fig. 1, etc. The narrowing of the zone of precipitation as the concentration of dispersoid colloid is lowered, and the ultimate disappearance of a zone of mutual coagulation in the absence of electrolyte are clearly shown in Fig. 4; with very dilute sols the sensitising effect of the protective colloid will not manifest itself, and the relation between sensitisation and protection will be obscured. This may account for the results of Billitzer (*loc. cit.*) and other workers who did not observe precipitation by gelatin alone.

The results of Kermack and MacCullum (*loc. cit.*) give a diagram somewhat similar to that found in the present work when hydrogen ion is regarded as the precipitating electrolyte. From p_H 4.8 to p_H 13 no coagulation was found, and from p_H 13 to p_H 15 a zone of coagulation by alkalis occurred, which, of course, has no counterpart in our curves. These workers found also that sodium chloride broadened the zone of mutual coagulation of the two colloids, which is again in agreement with our results.

The types of curve obtained in these experiments suggest at once a comparison with the results of Billitzer (*loc. cit.*) on the mutual precipitation of oppositely charged colloids when present in amounts sufficient to neutralise one another. The precipitation of both positive and negative sols by gelatin might then be ascribed to the amphoteric character of this colloid (compare Rideal, *loc. cit.*). We suggest this explanation tentatively, but intend to examine other systems and to study the sign of the electrical charge in the neighbourhood of the zone of coagulation in order to test this hypothesis. In particular, the characteristic bulge or proboscis which occurs in all the diagrams seems to indicate that some factor other than simple neutralisation of the sol is concerned.

*Summary.**

(1) Simultaneous variation of the concentrations of protective colloid and electrolyte in a series of systems, dispersoid colloid-protective colloid-electrolyte, has revealed an area of coagulation of characteristic shape.

(2) In dilute solutions, protective colloids sensitise the sol and at suitable concentrations cause precipitation in the absence of more than traces of electrolytes.

(3) For arsenious sulphide sols with concentrations of less than 1 g. per litre the amount of gelatin which causes maximum precipitation is proportional to the arsenious sulphide content.

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